Remarks

We note the withdrawal of Claims 7 - 11 in response to the Applicants' earlier election of Claims 1 - 6. Claims 7 - 11 have accordingly been canceled.

We note with appreciation the Examiner's helpful suggestion with respect to a new title and have adopted that suggested title.

The Specification has been amended to correct minor typographical and grammatical errors.

We have amended Claim 1 in accordance with the Examiner's helpful suggestion to remove reference to "plural" in favor of "various types of". Withdrawal of the 35 U.S.C. §112 second paragraph rejection is accordingly respectfully requested. We have also amended Claim 2, inasmuch as it also contains "plural".

Claim 1 has further been amended to recite that the disease under examination is a pruritic disease. Accordingly, Claim 5 has been canceled. Claim 6 has been amended in view of the cancellation of Claim 5 and now depends on Claim 1. Withdrawal of the 35 U.S.C. §112 first paragraph rejection of Claims 1 - 4 is respectfully requested.

We respectfully submit that the 35 U.S.C. §102 rejections of Claims 1 - 3, based on Simonnet, and Claim 1, based on Ripamonti, are now moot in view of the amendment to Claim 1 specifying that the method for examining a disease refers to a pruritic disease.

In light of the foregoing, we respectfully submit that the entire Application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,

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In the Title (Clean Copy)

METHOD FOR EXAMINING THE INVOLVEMENT OF OPIOID PEPTIDES IN PRURITIS

In the Specification (Clean Copy)

On page 1, please replace the second paragraph with the following:

Recently, it has become evident that opioids have a wide variety of biological activities. For examples, opioids are not only involved in pain, but also act upon the immune system (T. Hosokawa et al., Pain Clinic, 21, 857-864, 2000). However, roles which the opioids play in vivo have not been sufficiently clarified other than those in pain. For example, the relation of the opioids with itching (pruritus) is now being clarified, while itching is considered to be close to pain.

A 1

On page 2, please replace the first full paragraph with the following:

For pruritus therapy, antihistaminic agents or antiallergic agents are mainly used as oral medicines, and antihistaminic agents, adrenocortic steroidal medicines for external application, nonsteroidal antiphlogistics, camphor, menthol, phenol, salicylic acid, rectified tar oil, crotamiton, capsaicin, moisture-retentive agents (e.g., urea, Hirudoid (trade name; a heparinoid from animal organs, available from Maruho Co., Ltd.), and Vaseline). Of these therapeutic agents, only antihistaminic agents against urticaria are accepted to have a sufficient therapeutic efficacy. Other agents are not always sufficient in therapeutic efficacy in many cases (Y. Miyaji, Itching Q & A, p. 20, 1997, Iyaku (Medicine and Drug) Journal Co., Ltd.). The reason for this phenomenon is that inflammatory mediators such as histamine have been considered to be a main cause of itching, and antihistaminic agents and antiallergic agents serving as antagonists against these mediators have therefore been developed as therapeutic agents for pruritus.

A 2

Please replace the paragraph spanning pages 2 and 3 with the following:

At present, histamine, serotonin, substance P, bradykinin, proteinases, prostaglandins, and opioid peptides are known as stimulators to initiate itching. The itching sensation is considered to be initiated by the following mechanism. Specifically,

A 3

itching stimulators act upon nerve endings (itching receptors) which reside in the epidermis-dermis interface, are composed of C fibers and are responsible to multi-stimulation. The resulting impulses are transmitted to afferent C fibers and reach the spinothalamic tract, thalamus and cerebral cortex in this order to induce itching (Y. Miyaji; Approach to Treatment of Skin Pruritus, p. 22, 1996, Sentan-Igaku (Advanced Medicine) Co Ltd.).

A3

Please replace the paragraph spanning pages 3 and 4 with the following:

Some opioids have been known to be involved in inducement of itching. For example, it has been reported that endogenous opioids such as β-endorphin and enkephalin induce itching (B. Fjeller; Acta, Dermato-Venereol., 61 (suppl. 97), 1-34, 1981). In addition, it has been clarified that the epidural or intraspinal administration of morphine or an opioid compound initiates itching as an adverse drug reaction (J. H. Jaffe and W. R. Martin; Goodman and Gilman's Pharmacological Basis of Therapeutics, Macmillan, New York, 1985). On the other hand, it has been clarified that itching initiated by the intraspinal administration of morphine is inhibited by naloxone, a morphine antagonist (J. Bernstein et al.; J. Invest. Dermatol., 78, 82-83, 1982) and that a severe itching in a patient with hepatopathic cholestasia, which is firmly suggested to be induced by an increased enkephalin, is inhibited by an opioid antagonist nalmefene (J. R. Thornton and M. S. Losowsky; Br. Med. J., 297, 1501-1504, 1988). It is therefore the consensus view that opioid agonists act to initiate itching and, in contrast, opioid antagonists have an antipruritic activity. In addition, it has been reported that the serum concentrations of β-endorphin in children with atopic dermatitis are significantly higher than those in healthy children and that opioid antagonists may be effective against itching (S. Georgala et al.; J. Dermatol. Sci., 8, 125-128, 1994).

A4

Please replace the paragraph spanning pages 4 and 5 with the following:

As such opioid receptors, μ-, κ- (kappa), δ- (delta), and ORL-1 (nociceptin) receptors are known, and endogenous opioid peptides that respectively selectively stimulate individual receptors have been found. For example, μ- and δ-receptor agonistic endogenous opioid peptides include β-endorphin and enkephalin, respectively, and κ-receptor agonistic endogenous opioid peptides include dynorphin, and ORL-1 receptor agonistic endogenous opioid peptides include nociceptin. Of these opioid peptides, β-endorphin is known as an opioid peptide which possibly invites itching. In contrast, κ-opioid agonists have been found to inhibit itching in recent years (PCT International Publication No. 98/23290), suggesting a possibility that κ-receptor agonistic endogenous peptides inhibit itching.

Please replace the paragraph spanning pages 5 and 6 with the following:

When opioids are involved in the itching pathosis, it is supposed that μ-opioids (μ-opioid peptides or expression of μ-opioid receptors) are facilitated more than κ-opioids (κ-opioid peptides or expression of κ-opioid receptors). The μ-opioids have the possibility of inviting itching, and the κ-opioids have the possibility of suppressing itching. We intensively investigated a concept that the relation between opioids and itching can be clarified by measuring the concentrations of opioids or degrees of expression of opioid receptors in blood cell, body fluid, or tissue to thereby verify that μ-agonistic substances have the advantage over κ-opioid agonistic substances or that μ-opioid receptors have the advantage over κ-opioid receptors. The present invention has been accomplished on the basis of these investigations and resulting findings.

Please replace the paragraph spanning pages 7 and 8 with the following:

According to the present invention, whether opioids are involved in a disease is determined by measuring the concentrations of plural opioids in blood cells, body fluid,

or tissue and calculating the ratio of the concentrations, or by measuring the degrees of expression of plural opioid receptors and comparing the degrees of expression. Specifically, whether opioids are involved in a disease is determined by measuring the concentrations of plural opioids selected from μ-, κ-, δ-, and ORL-1 receptor agonistic endogenous peptides in sampled blood cells, body fluid, or tissue and determining the ratio of the concentrations, or by measuring degrees of expression of plural opioid receptors selected from μ-, κ-, δ-, and ORL-1 opioid receptors and comparing the measured degrees of expression.

A7

On page 9, please replace the first full paragraph with the following:

The concentrations of opioid peptides in body fluids can be measured by, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (EIA) or chromatography. The concentrations of opioid peptides in blood cells or tissue can be measured by extracting target substances from blood cells or tissue and measuring the concentrations through any of the above assays or by extracting total RNA or mRNA from blood cells or tissue and measuring the expression of peptide molecules by RT-PCR (reverse transcriptase-PCR) or other molecular-biological techniques. Such radio-immunoassay, enzyme-linked immunosorbent assay, chromatography, RT-PCR, and other techniques for use in the measurement can be performed in a conventional manner.

A8

On page 10, please replace the second paragraph with the following:

The degrees of expression of opioid receptors are preferably measured by a process of extracting total RNA or mRNA in a specimen and measuring the degrees through RT-PCR method (reverse-transcriptase-PCR method) or other molecular-biological techniques. The degrees of expression of receptor molecules can also be measured by an immunochemical technique using antibodies against the receptors or by the receptor binding method using labeled ligands for individual receptors. For example, in the

A9

measurement by the RT-PCR method, the degrees of expression of receptors can be scored using intensities of bands obtained through electrophoresis as indexes. The RT-PCR method, immunochemical technique using antibodies against receptors, receptor binding method, and other techniques for use in the measurement can be performed in a conventional manner.

A9

Please replace the paragraph spanning pages 10 and 11 with the following:

The concentrations of opioid peptides and the degrees of expression of opioid receptors can be measured by any technique as far as they can qualitatively or quantitatively measure the expression of opioid receptors substantially, and they are not limited to the aforementioned techniques.

A10

Please replace the paragraph spanning pages 11 and 12 with the following:

For example, when the involvement of opioids in pruritus is to be determined, the ratio of the concentrations of different opioid peptides is calculated or the degrees of expression of different opioid receptors are compared with each other in cases with itching, to thereby verify that a μ-agonistic opioid peptide has the advantage over a κ-agonistic opioid peptide in blood cells, body fluid, or tissue or that the degree of expression of a μ-opioid receptor has the advantage over a κ-opioid receptor. Examples of indications for the determination are that the plasma concentration of a μ-agonistic opioid peptide is higher than that of healthy adults, that the concentration of a μ-agonistic opioid peptide is relatively higher than that of healthy adults when the concentration of a κ-agonistic opioid peptide and that of the μ-agonistic opioid peptide are compared, and that the representation of a μ-agonistic opioid peptide in tissue is relatively higher than that of healthy adults when the representation of the μ-agonistic opioid peptide and that of a κ-agonistic opioid peptide are compared. Regarding the opioid receptors, an indication for determination is, for example, that the degree of expression of a μ-receptor in blood cell component or in tissue

A11

is relatively higher than that of a κ-receptor as compared with healthy adults. In addition, whether opioids are involved in itching can be comprehensively determined by considering the concentrations or the ratio thereof of endogenous opioid peptides in blood cells, body fluid, or tissue, as well as the degrees of expression of opioid receptors in blood cells, body fluid, or tissue.

On page 16, please replace the first paragraph with the following:

Human μ-receptor stable expression cells (Mu/CHO) and human κ-receptor stable expression cells (Kappa/CHO) were prepared, as positive control cells, according to a conventional technique. Separately, CHO cell was transformed only with an expression vector pCR3 to yield a transformed CHO cell ((-)/CHO) as a negative control cell. These cells were respectively cultivated in a nucleic acid-containing minimum essential medium α (α-MEM) (available from GIBCO, BRL) containing 10% (v/v) fetal calf serum (available from Biological Industries) at 37°C in the presence of 5% CO_2.

On page 16, after the first paragraph, please replace the following heading:

(b) Recovery of Cells

On page 18, please replace the first full paragraph with the following:

Initially, total RNA was extracted from the positive control Mu/CHO or Kappa/CHO and was subjected to RT-PCR, which also served as examination of the optimum amount of RNA in an RT reaction. In combinations of primers pMA1/pMA4 and pKA1/pKA2, PCR was performed for 30 cycles of cyclic reactions comprising a thermal denaturation reaction at 95°C for 30 seconds, an annealing reaction at 65°C for 30 seconds, and an elongation reaction at 72°C for 30 seconds, in this order. In combinations of primers pMA2/pMA3 and pKB1/pKB2, PCR was performed for 30 cycles of cyclic reactions comprising a thermal denaturation reaction at 95°C for 30 seconds, an annealing reaction at 55°C for 30 seconds, and an elongation reaction at 72°C for 30

seconds, in this order. In the reaction, DNA of a target size was observed as a single band as a result of DNA amplification as shown in Fig. 3, indicating that the expression of each receptor in the positive control can be analyzed. The symbol "M" in the figure represents a DNA size marker. Each band constitutes a 100-bp ladder. The density of each band did not differ with varying RNA amount, and the amount of the template total RNA in the following procedures was therefore set at 1 μg, which is a conventionally employed amount. In the RT-PCR, no DNA amplification was observed when RNA of the negative control (-)/CHO was used as a template.

A14

Please replace the paragraph spanning pages 22 and 23 with the following:

Based on the results in the above (1) and (2), four patients were selected from a group to which conventional antihistaminic agents and antiallergic agents were inefficient and who constantly complained of severe itching. One capsule of a κ-opioid agonist 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-(N-methyl-trans-3-(3-furyl)acrylamide) morphinan hydrochloride capsule (10 μg/body) was administered to each of the selected patients. In all the administered patients, itching was relieved after two hours from the administration, and the antipruritic effect continued until 24 hours from the administration. To quantitatively determine the severity of itching, visual analogue scale (VAS) was employed, in which the severity of itching was determined on a straight line of 0 to 100 mm where no itching was rated as "0" and the supposable most severe itching was rated as "100". As a mean of the four subjects, the VAS was 75 mm before administration but it was 0 mm after 4 to 24 hours from the administration.

A15

In the Claims (Clean Copy)

1. (Amended) A method for examining a pruritic disease, comprising:

measuring concentrations of various types of opioid peptides in blood cells, body fluid, or tissue, and

calculating the ratio of said concentrations to thereby determine whether opioids are involved in the disease.

2. (Amended) A method according to claim 1, wherein said opioid peptides to be measured are opioid peptides selected from the group consisting of a μ-opioid peptide, a κ-opioid peptide, a δ-opioid peptide, and nociceptin.

6. (Amended) A method according to claim 1, wherein said pruritic disease is pruritus with atopic dermatitis, neurodermatitis, contact dermatitis, seborrheic dermatitis, autosensitization dermatitis, caterpillar dermatitis, asteatosis, senile pruritus, insect bite, hyperesthesia optica, urticaria, prurigo, herpes, impetigo, eczema, tinea, lichen, psoriasis, scbies, acne vulgaris, malignant tumor, diabetes, hepatic disease, renal failure, hemodialysis, peritoneal dialysis, or pregnancy.

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